Applicants: Levy, et al. Docket No.: 16534-539001US

Serial No.: 10/692,764 Filing Date: October 24, 2003

Page -2-

AMENDMENTS TO THE CLAIMS:

Listing of Claims:

This listing of claims will replace all prior versions of the claims and listing of the claims in the application:

1. (Currently Amended) A method for treating a subject for a disease treatable by modulation of RNA (DTMR) associated with splicing of nuclear RNA, comprising: administering to said subject an effective amount of a tetracycline compound of formula (I):

wherein

R², R², R⁴, and R⁴ are each independently hydrogen[[,]] or alkyl, alkenyl, alkynyl, alkylylthio, alkylsulfinyl, alkylsulfonyl, alkylamino, arylalkyl, aryl, heterocyclic, heteroaromatic or a prodrug moiety;

R³, R¹⁰, R¹¹ and R¹² are each hydrogen, alkyl, alkenyl, alkynyl, substituted carbonyl, or a pro-drug moiety;

R⁴ is NR⁴'R⁴", alkyl, alkenyl, alkynyl, hydroxyl, halogen, or hydrogen;

R⁵ is hydroxyl, hydrogen, thiol, alkanoyl, aroyl, alkaroyl, aryl, heteroaromatic, alkyl, alkenyl, alkoxy, alkylthio, alkylsulfinyl, alkylsulfonyl, alkylamino, arylalkyl, alkyl carbonyloxy, or aryl carbonyloxy;

R⁶ and R⁶ are each independently-hydrogen, absent, hydroxyl, halogen, thiol, alkyl, alkenyl, alkynyl, aryl, alkoxy, alkylthio, alkylsulfinyl, alkylsulfonyl, alkylamino, or an arylalkyl;

R⁷ is hydrogen, hydroxyl, halogen, thiol, nitro, alkyl, substituted alkenyl, substituted alkynyl, arylsubstituted phenyl, substituted or unsubstituted furanyl, alkoxy,

Docket No.: 16534-539001US

Applicants: Levy, *et al.* Serial No.: 10/692,764 Filing Date: October 24, 2003

Page -3-

alkylthio, alkylsulfinyl, alkylsulfonyl, arylalkyl, amino, arylalkenyl, arylalkynyl, acyl, <u>or</u> aminoalkyl, heterocyclic, thionitroso, or <u>(CH₂)_{0.3}NR^{7e}C(=W')WR^{7a}</u>;

R⁸ is hydrogen, hydroxyl, halogen, thiol, nitro, alkyl, alkenyl, alkynyl, aryl, alkoxy, alkylthio, alkylsulfinyl, alkylsulfonyl, alkylamino, amino, arylalkenyl, arylalkynyl, acyl, aminoalkyl, heterocyclic, thionitroso, or (CH₂)_{0.3}NR^{8e}C(=E')ER^{8a};

 R^9 is hydrogen, hydroxyl, halogen, thiol, nitro, alkyl, alkenyl, alkynyl, aryl, alkoxy, alkylthio, alkylsulfinyl, alkylsulfonyl, arylalkyl, amino, arylalkenyl, arylalkynyl, aeyl, aminoalkyl, heterocyclic, thionitroso, or $-(CH_2)_{0.3}NR^{9e}C(=Z^2)ZR^{9a}$; and

R^{7a}, R^{7b}, R^{7e}, R^{7d}, R^{7e}, R^{7f}, R^{8a}, R^{8b}, R^{8e}, R^{8d}, R^{8e}, R^{8f}, R^{9a}, R^{9b}, R^{9e}, R^{9d}, R^{9e}, and R^{8f}-are each independently hydrogen, acyl, alkyl, alkenyl, alkynyl, alkoxy, alkylthio, alkylsulfinyl, alkylsulfonyl, alkylamino, arylalkyl, aryl, heterocyclic, heteroaromatic or a prodrug moiety;

R¹³-is hydrogen, hydroxy, alkyl, alkenyl, alkynyl, alkoxy, alkylthio, aryl, alkylsulfinyl, alkylsulfonyl, alkylamino, or an arylalkyl;

E is CR^{8d}R^{8e}, S, NR^{8b}-or O;

E' is O, NR^{8f}, or S;

W is CR^{7d}R^{7e}, S, NR^{7b}-or O;

W' is O, NR^{7f}, or S;

X is CHC(R¹³Y'Y), CR^{6'}R⁶, S, NR⁶, or O;

Y' and Y are each independently hydrogen, halogen, hydroxyl, cyano, sulfhydryl, amino, alkyl, alkenyl, alkynyl, alkoxy, alkylthio, alkylsulfinyl, alkylsulfonyl, alkylamino, or an arylalkyl;

Z' is O, S, or NR 9f, or a pharmaceutically acceptable salt, ester or enantiomer thereof, with the proviso that said tetracycline compound is not tetracycline;

such that said DTMR associated with splicing of nuclear RNA is treated, wherein said DTMR associated with splicing of nuclear RNA is spinal muscular atrophy, and further

Applicants: Levy, *et al.*Serial No.: 10/692,764

Docket No.: 16534-539001US

Filing Date: October 24, 2003

Page -4-

wherein said effective amount is effective to modulate splicing of said subject's nuclear RNA.

2.-36. (Canceled)

37. (Previously Presented) The method of claim 1, wherein $R^2[[,]]$ and R^2 , R^8 , R^{10} , R^{11} , and R^{12} are each hydrogen, X is CR^6R^6 , and R^4 is NR^4 , wherein and R^4 and R^4 are each methyl.

38. (Canceled)

- 39. (Currently Amended) The method of claim 38 claim 37, wherein R⁷ is substituted or unsubstituted arylfuranyl.
- 40. (Currently Amended) The method of elaim 39 claim 37, wherein R⁷ is substituted or unsubstituted phenyl.

41. (Canceled)

42. (Currently Amended) The method of elaim 41claim 40, wherein said substituted phenyl is substituted with one or more substituents and further wherein said substituents are each independently alkyl, alkenyl, alkynyl, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonato, phosphinato, cyano, amino, acylamino, amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, alkylsulfinyl, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkylaryl, aryl or heterocyclic moiety.

Applicants: Levy, et al. Docket No.: 16534-539001US

Serial No.: 10/692,764 Filing Date: October 24, 2003

Page -5-

43. (Currently Amended) The method of claim 38 claim 37, wherein R⁷ is substituted or unsubstituted alkenyl.

- 44. (Currently Amended) The method of claim 37, wherein R⁷ is substituted <u>alkynylor unsubstituted heteroaryl and R⁹ is alkyl</u>.
- 45. (Currently Amended) The method of claim 36 claim 37, wherein R⁷ is dialkylaminoacyl.
- 46. (Currently Amended) The method of claim 45 claim 37, wherein $\mathbb{R}^9 \underline{\mathbb{R}^7}$ is alkylaminoaminoalkyl.

47.-56. (Canceled)

57. (Previously Presented) The method of claim 1, wherein said tetracycline compound is:

58. (Canceled)

59. **(Previously Presented)** The method of claim 1, wherein said modulation of splicing increases splicing of RNA.

Applicants: Levy, et al. Docket No.: 16534-539001US

Serial No.: 10/692,764 Filing Date: October 24, 2003

Page -6-

60. (Previously Presented) The method of claim 1, wherein said modulation of splicing decreases splicing of RNA.

61. (Canceled)

- 62. (Previously Presented) The method of claim 1, wherein said subject is a mammal.
- 63. (Previously Presented) The method of claim 62, wherein said mammal is a human.
- 64. (Previously Presented) The method of claim 1, wherein said modulation of splicing is activation of cryptic splice sites, silencing of consensus splice sites, silencing of exonic or intronic splicing enhancers (ESEs or ISEs), silencing of exonic or inronic splicing silencers (ESSs or ISSs), alteration of the binding or a component of the splicing machinery to the RNA, or the affecting of intermolecular interactions between components of the splicing machinery.
- 65. (Previously Presented) The method of claim 1, wherein said tetracycline compound is:

, or a pharmaceutically acceptable salt thereof.

Applicants: Levy, *et al.* Serial No.: 10/692,764 Filing Date: October 24, 2003

Page -7-

66. (New) A method for treating a subject for a DTMR associated with splicing of nuclear RNA, comprising: administering to said subject an effective amount of a tetracycline compound; wherein said tetracycline compound is a tetracycline compound selected from the group consisting of:

Applicants: Levy, et al. Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -8-Docket No.: <u>16534-539001US</u>

Applicants: Levy, et al. Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -9-

Applicants: Levy, et al. Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -10-

Applicants: Levy, et al. Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -11-

Applicants: Levy, et al. Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -12-

Applicants: Levy, et al. Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -13-

Applicants: Levy, et al. Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -14-

Docket No.: <u>16534-539001US</u>

Applicants: Levy, et al. Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -15-

Docket No.: 16534-539001US

Applicants: Levy, et al. Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -16-

Applicants: Levy, et al. Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -17-

Applicants: Levy, et al. Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -18-

Docket No.: <u>16534-539001US</u>

Applicants: Levy, et al. Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -19-Docket No.: 16534-539001US

Applicants: Levy, et al. Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -20-

Applicants: Levy, et al. Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -21-

Applicants: Levy, et al. Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -22-

Applicants: Levy, *et al.* Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -23-

Applicants: Levy, et al. Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -24-

Applicants: Levy, *et al.* Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -25-

Applicants: Levy, *et al.* Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -26-

Applicants: Levy, et al. Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -27-

Applicants: Levy, et al. Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -28-

Applicants: Levy, et al. Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -29-

Applicants: Levy, et al. Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -30-

Applicants: Levy, et al. Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -31-

Applicants: Levy, et al. Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -32-Docket No.: <u>16534-539001US</u>

Applicants: Levy, et al. Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -33-

Docket No.: <u>16534-539001US</u>

Applicants: Levy, *et al.* Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -34-

Applicants: Levy, *et al.* Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -35-

Applicants: Levy, et al. Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -36-Docket No.: <u>16534-539001US</u>

Applicants: Levy, *et al.* Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -37-

Applicants: Levy, et al. Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -38-

$$\begin{array}{c} \begin{array}{c} \begin{array}{c} \\ \\ \\ \\ \\ \\ \end{array} \end{array}$$

Applicants: Levy, et al. Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -39-

$$\begin{array}{c} \begin{array}{c} \begin{array}{c} \\ \\ \\ \\ \\ \\ \\ \\ \\ \end{array} \end{array}$$

Applicants: Levy, et al. Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -40-

Applicants: Levy, et al. Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -41-

Applicants: Levy, et al. Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -42-

Applicants: Levy, et al. Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -43-

Applicants: Levy, et al. Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -44-

Docket No.: 16534-539001US

Applicants: Levy, et al. Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -45-

Applicants: Levy, et al. Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -46-

Applicants: Levy, et al. Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -47-

$$\begin{array}{c|c} F & \underline{H} & \underline{H} & \underline{N} \\ \hline \\ OH & OH & O \\ \end{array}$$

$$\begin{array}{c|c} CH_2 & \underline{H} & \underline{N} \\ \hline \\ OH & O & OH & O \\ \end{array}$$

Applicants: Levy, et al. Serial No.: 10/692,764 Filing Date: October 24, 2003 Page -48-

$$\begin{array}{c|c} O & H & \underline{H} & \underline{N} \\ O & \underline{H} & \underline{H} & \underline{N} \\ \hline O & O & O \\ \hline O & O & O \\ \end{array}$$

$$\begin{array}{c|c} & & & \\ & & \\ & & \\ \hline \\ OH & O & OH \\ \hline \\ OH & O \\ \end{array}$$

$$\begin{array}{c|c} & & & \\ & & \\ N & & \\ \hline N & \\ N & \\ \hline OH & \\ OH & \\ OH & \\ OH & \\ OH \\ \end{array}$$

$$\begin{array}{c|c} & & & \\ & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & &$$

$$\begin{array}{c|c} & H & \underline{H} & \underline{N} \\ N & \underline{H} & \underline{H} & \underline{N} \\ \hline OH & OOH & OOH \\ \end{array}$$

Applicants: Levy, *et al.* Serial No.: 10/692,764 Filing Date: October 24, 2003

Page -49-

Docket No.: <u>16534-539001US</u> Applicants: Levy, et al.

Serial No.: 10/692,764

Filing Date: October 24, 2003

Page -50-

and pharmaceutically acceptable salts, esters and enantiomers thereof; such that said DTMR associated with splicing of nuclear RNA is treated, wherein said DTMR associated with splicing of nuclear RNA is spinal muscular atrophy, and further wherein said effective amount is effective to modulate splicing of said subject's nuclear RNA.